Use of Non-Native Macroalgal Habitat by Hatchery-Reared and Wild Blue Crab Juveniles

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by

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ABSTRACT

Seagrass beds are considered the preferred nursery habitat for juvenile blue crabs, *Callinectes sapidus,* increasing both survival and growth in early juvenile stages. Degradation of this structured nursery habitat and a drastic decline in spawning stock, due to natural and fishing mortality, has many scientists concerned about the Chesapeake Bay's blue crab population. This study aimed to determine whether non-native macroalgae, *Gracilaria* spp., may function as an alternative nursery habitat for juvenile crabs and whether *Gracilaria* spp. may help to increase release success of hatchery-reared cohorts as part of stock enhancement efforts. In this study, ~28,000 hatchery-reared blue crab juveniles (mean size 7.15mm carapace width-CW) were released near the mouth of the York River in an unvegetated mud cove enhanced with ~3600 L of *Gracilaria* spp. Sampling was conducted in two areas of the cove using a basket apparatus. The number and size (carapace width-CW) of crabs were measured during each sampling. The crabs collected during sampling were identified as hatchery-reared or wild using genetic analysis. Crab density at each site suggests *Gracilaria* in the mud cove had a carrying capacity of ~4-8 crabs m^{-2}. Genetic analysis determined that some hatchery-reared crabs remained within the mud cove for the entire 43 day study period. Mean carapace width for the hatchery-reared cohort increased from 7.15mm (SE+/- 0.0581) to 26.6mm (SE+/- 1.93). In addition, settlement of wild juvenile recruits in *Gracilaria* was observed in early August. These findings suggest that the non-native macroalgae, *Gracilaria* spp., serves as an alternative nursery habitat for blue crab juveniles and release of hatchery-reared juveniles into habitats containing *Gracilaria* may help to increase post-release success.

INTRODUCTION

The blue crab, *Callinectes sapidus*, is an important part of the ecology and economy of the Chesapeake Bay. In recent decades, habitat degradation and continued fishing pressure have resulted in dramatic declines in the bay's blue crab abundance (Bunnell and Miller, 2005; Bi-State Blue Crab Technical Advisory Committee BBCTAC, 2006; Lipcius et al. 2005b). These declines have prompted efforts to protect and restore blue crab habitat and enhance the bay's spawning stock. This study sought to evaluate the role of the non-native macroalgae, *Gracilaria* spp,. in aiding both of these efforts.

Role of Habitat in the Blue Crab Life History

Habitat selection plays an important role in the blue crab life history. Blue crab megalopae (9^{th} larval stage) recruit into the Chesapeake Bay and preferentially settle in seagrass beds (primarly *Zostera marina*) using both chemical and structural cues (Orth and van Montfrans 1987; van Montfrans 2003; Welch 1997). Following settlement, megalopae metamorphose into the first benthic juvenile stage (Orth and van Montfrans 1987). Seagrass habitats are a vital source of food and refuge increasing both survival and growth in early juveniles (1^{st} through 7^{th} instars (20-30mm CW)) (Heck et al., 2003). The current paradigm of habitat use postulates that juveniles >20mm CW disperse from primary structured habitats into secondary shallow unstructured habitats as they outgrow the protection of seagrass structure and become less susceptible to predation by gape-limited predators present in secondary habitats (i.e. mud and sand flats; Pile et al., 1996; Lipcius et al. 2007). These unvegetated habitats also offer ample prey allowing juveniles to maximize their growth (Seitz et al., 2005). Density-dependent emigration by smaller

juveniles (<20mm CW) to alternative structured and unstructured habitats also occurs to avoid predation by conspecifics (Reyns and Eggleston, 2004).

Increasing fragmentation and decreasing density of seagrass habitats in the Chesapeake Bay is of particular concern given the importance of this structured habitat for settlement and dispersal of blue crab larvae and juveniles. Landscape-level changes in spatial patterns of seagrass habitat have the potential to negatively affect recruitment and settlement of blue crab larvae (Stockhausen & Lipcius, 2003). Concern about the loss of this critical nursery habitat has prompted conservation and restoration efforts. The limited success of these efforts has many wondering if other substrates may serve as alternatives to seagrass habitats. It has been suggested that other structurally complex habitats, such as oyster reefs and macroalgae, may function similarly to seagrass beds (Heck et al., 2003, Wilson et al., 1990, Epifanio et al., 2003) but further research is needed to assess the ability of these substrates to function as alternative nursery habitats.

Fisheries Management and Stock Enhancement Efforts

In addition to environmental degradation, extensive exploitation by the blue crab fishery has added further stress to crab populations in the Chesapeake Bay (Bunnell & Miller, 2005). Of particular concern are the impacts of exploitation on the spawning stock. A study spanning from 1988-2002 showed an 84% decline in spawning stock abundance (Lipcius & Stockhausen, 2002). This was correlated with a one order of magnitude decrease in larval abundance and post-larval recruitment. These findings demonstrated the increased risk of recruitment failure and the immediate need for conservation efforts to ensure persistence of the bay's blue crab population. Stringent management policies put in place in 2001, as well as the enlargement of the now

240,092ha. spawning sanctuary in the lower Bay, have helped to stabilize the decline of the bay's blue crab population; however, spawning stocks remain at low levels (BBCTAC 2006).

The combination of a drastically depleted spawning stock, declining juvenile abundance, and evidence indicating many nursery habitats are far below carrying capacity (Hines et al., 2008; Seitz et al., 2008) has suggested that the Chesapeake Bay blue crab may be a viable candidate for stock enhancement. In 2001, the Blue Crab Advanced Research Consortium (BCARC) was established with the primary goals of advancing the understanding of blue crab biology and assessing the feasibility of a stock enhancement program to replenish the blue crab breeding stock (Zohar et al., 2008). Since then, 290,000 hatchery-reared blue crabs have been released in nursery areas throughout the bay region (Zohar et al., 2008). Early results of these studies have been encouraging and efforts continue to maximize the efficiency of hatchery production.

The ability of released, hatchery-reared juveniles to survive and grow to maturity is crucial to the success of the stock enhancement effort. Thus, several studies have been conducted to evaluate tactics to enhance performance and release of hatchery-reared crabs. Hatchery-reared blue crabs have been found to differ both morphologically and behaviorally from their wild counterparts (Davis et al. 2004, 2005a); however, these differences have not been found to result in reduced survival post-release (Young et al., 2008).

Considerable work has also been conducted to determine release strategies that maximize growth and survival of hatchery-reared blue crabs (Johnson et al., 2008; Hines et al., 2008). Crab size at release, season of release, habitat of release site, and stocking

densities have all been identified as important variables (Johnson et al., 2008, Hines et al., 2008). Field tethering studies show survival of hatchery-reared crabs increases with size up to 40mm CW, suggesting release success may increase with size of individuals (Johnson et al., 2008). Survival to maturity of released hatchery-reared cohorts has been shown to be highest in the spring and fall. There also appears to be an interactive effect between release season and size; release size has been shown to have less of an effect on survival in the spring than in the fall (Johnson et al. 2008). Spring releases appear to be particularly favorable as cohorts released at this time have been able to grow to maturity and mate during their first year, while cohorts released in the fall must overwinter and do not reach maturity until the following year (Johnson et al., 2008; Hines et al, 2008; Zohar et al., 2008). Spring releases may also prevent competition between hatchery cohorts and wild juveniles during the peak recruitment period (Johnson et al. 2008).

Release habitat is also an important factor in increasing release success (Hines et al. 2008). Essential attributes of release habitat are food availability, predator abundance, and available structure for refuge (Hines et al., 2008). Salt-marsh fringed mud coves are a major habitat of juvenile blue crabs in much of the bay area (Seitz et al., 2008; King et al., 2005); however, survival of released hatchery cohorts has been quite variable in these environments (Hines et al., 2008). Further research will be needed to improve the ability to identify optimal habitats for release of hatchery-reared cohorts.

Role of Macroalgae

Eutrophication has simultaneously decreased seagrass coverage and increased macroalgal blooms worldwide (Duarte 1995; Burkholder et al., 2007). Macroalgae often colonizes bottom made available by retreating seagrass beds (Valiela et al., 1997). These

factors are likely responsible for the ubiquity of *Gracilaria* spp., a non-native, structurally complex, red macroalgae, in many regions of the Chesapeake Bay. Despite its prominence, relatively little is known about the ecological role *Gracilaria* plays in the bay area. Observations suggest this macroalgae is a suitable habitat for juvenile blue crabs, increasing both survival and growth of juveniles relative to unvegetated habitat (Johnston et al., unpublished). A study in Rehoboth Bay found that *Gracilaria* supported juvenile crab abundance equal to seagrass meadows (Epifanio et al., 2003). Further study of *Gracilaria* as a potential blue crab nursery habitat in the Chesapeake Bay is clearly warranted. Additionally, *Gracilaria* often occurs in habitats optimal for release of hatchery cohorts (e.g. salt-marsh-fringed mud coves), making it an important factor to consider when assessing release strategies.

The goals of this study were to test the ability of *Gracilaria* spp. to promote the post-release settlement and growth of hatchery-reared blue crabs and to evaluate the carrying capacity of this macroalgae for blue crab juveniles. It was postulated that the complex structure of *Gracilaria* mats would support abundances of juvenile crabs comparable to seagrass and that presence of this substrate would increase settlement of hatchery-reared cohorts at the release site. To test these hypotheses, both abundance and size of released hatchery-reared, as well as wild, blue crab juveniles in *Gracilaria* were measured over a forty-three day period in a salt-marsh fringed mud cove near the mouth of the York River. This study also sought to assess the use of genetic markers in tracking hatchery-reared crabs following their release (as opposed to using microwire tags). All crabs collected during sampling were sent to the Center of Marine Biotechnology (COMB) for genetic identification.

MATERIALS AND METHODS

Hatchery-reared crabs were transported from the Piney Point hatchery in St. Mary's County, MD to the Virginia Institute of Marine Science (VIMS) two days prior to their release. These crabs were held in outdoor tanks which circulated water from the York River. An estimated size distribution of the released hatchery-reared crabs was obtained from a sample of 400 individuals the day of the release (Fig. 3)

The field study was conducted in an unvegetated mud cove near the mouth of the York River estuary (Fig. 2). The back end of the cove was enhanced with ~3600L of *Gracilaria* spp. collected off the Goodwin Islands and distributed evenly across the back of the cove. Hatchery-reared juveniles (~28,000; mean size ~7mm CW) were released into the enhanced area of the cove on June 28, 2007.

Sampling was conducted in the cove every four to five days for a 43 day period. Sampling occurred in the following two areas of the cove: the region of the crab release near the back of the cove (release site) and an unvegetated region in the middle of the cove (unenhanced site). The purpose of sampling in the unenhanced site was to attempt to measure emigration of the hatchery-reared cohort out of the cove.

Basket Sampling

Sampling in the release and unenhanced site was conducted using a basket-like apparatus (Fig. 1). This apparatus was constructed from plastic tubing, filled with sand, and made into a hoop with a 56cm diameter. A fine 0.64cm mesh was attached around the bottom and large 15.2cm netting was secured over the top of the hoop with zip ties, creating a pocket to hold macroalgae. Four ropes were tied to the hoop and attached to a float to allow rapid retrieval. The baskets were deployed in the cove so that they lay flat

on the sediment bottom. Eighteen baskets were placed evenly throughout the release site and the unenhanced site, for a total of 36 baskets. Each basket contained one of three different volumes (400mL, 800mL, and 1600mL) of *Gracilaria* spp., for the purpose of examining the effect of algal volume on crab density. In the unenhanced site, which was largely unvegetated mud bottom, the baskets containing *Gracilaria* served as potential refugia for crabs, permitting estimation of numbers and size of dispersing hatchery crabs from the release site. Algal volume was measured by compressing algae in plastic containers of the appropriate volume.

Baskets were retrieved for sampling every four to five days and then randomly redeployed at the sites. When sampling, each basket was pulled up briskly using the float and ropes and immediately placed in a large sieve to be rinsed. Once rinsed, the basket was brought aboard a boat and the *Gracilaria* was removed from the basket. All crabs in the *Gracilaria* were counted, measured (carapace width-CW), and removed. After all crabs were removed, the *Gracilaria* was placed back in the basket and the basket was redeployed at the site. The collected crabs were stored in ethanol and sent to the Center of Marine Biotechnology (COMB) in Baltimore, Maryland where they were genetically identified as hatchery-reared or wild.

Suction Sampling

Crab ring suction sampling (Orth and van Montfrans, 1987) was conducted on three occasions; once prior to the release and twice following the release in both the *Gracilaria* enhanced area and unvegetated mud of the cove. On each occasion six suctions were conducted in each site. Suctioning was accomplished using a 1.67m^2 cylindrical mesh ring containing floats on top and weights on the bottom, allowing it to stand vertically in

the water column. A suctioning device was used to collect crabs within the ring in a 3mm mesh bag. Suctioning occurred for six minutes followed by four minutes of dip netting to remove crabs missed during the suction period. All crabs collected were counted and measured (CW).

Genetic Analysis

Genetic analysis was conducted by researchers at the Center of Marine Biotechnology in Baltimore, MD, for crabs collected on the first, seventh and ninth samplings (July 2, July 31, and Aug. 10). Crab DNA was extracted using Qiagen DNeasy(R) 96 Blood and Tissue Kit. PCR was performed with primers based on the blue crab mitochondrial NAD2 gene (Place et al. 2005). After purification, the PCR products were directly sequenced by using an ABI PRISM(R) 3100 Genetic Analyzer. Genotype comparison was done with the software Network4.5.0.0 Fluxus Technology Ltd. Only crabs with 685bp NAD2 fragment sequences identical to the female crabs producing specific batches were labeled hatchery crabs.

RESULTS

Pre-release hatchery-reared size composition

Mean carapace width (CW) for the sub-sample of hatchery-reared crabs on the release date was 7.15mm (SE+/- 0.0581). The smallest crab in the sub-sample was 3.1mm and the largest crab was 11.9mm CW (Fig. 3)

Basket sampling

The number of crabs collected in baskets did not vary significantly with algal volume (ANOVA, df=2, F=1.54, p=0.216) (Fig. 4), though there was an upward trend in density with higher algal volume. Additionally, crab abundance did not vary

significantly in either the release site (ANOVA, df=17, F=1.07, p=0.385) or the unenhanced site (ANOVA, df=17, F=0.71, p=0.784) according to basket.

Mean crab density within the unenhanced site remained fairly constant at ~7 crabs m^{-2} throughout the study period (Fig. 5), while in the release site, crab density declined from an initial density of ~44 crabs m^{-2} and leveled off around ~7 crabs m^{-2} after 22 days (Fig. 5). At both sites, crab density increased on the last two sampling dates due to an influx of wild recruits into the system (Fig. 5). Mean crab carapace width (CW) was much more variable in the unenhanced site than in the release site. At both sites, crab CW increased over time and decreased on the last two sampling dates, due to the influx of wild recruits (Fig. 6).

It was postulated that the trends in the observed mean crab density and mean crab size were likely attributable to the release of the hatchery-reared cohort within the area and that there would be no difference in the density of larger crabs at the two sites. To analyze this, mean density and mean carapace width were plotted over time for (1) crabs <25.9mm (Fig. 7a) and (2) crabs >25.9mm (Fig. 7b) in both sites. The cutoff of 25.9mm was selected because it sufficiently captured the hatchery-reared cohort without including larger wild crabs. The observed trends in mean crab density and mean crab CW in each site were found to be unique to crabs <25.9mm CW and absent in crabs >25.9mm CW in both sites, suggesting changes in density and crab size within the cove were due to changes in juvenile crabs. The number of crabs collected <25.9mm were significantly higher in the release site than in the unenhanced site for the first 18 days of the study (ANOVA, df=1, F=10.5, p=0.018), but the number of crabs collected <25.9 did not differ significantly between sites from the 22nd day onward (ANOVA, df=1, F=1.97,

p=0.198)(Fig 7a). The number of collected crabs >25.9mm did not vary significantly over time (ANOVA, df=8, F=1.84, p=0.190) or between the release and unenhanced sites (ANOVA, df=1, F=0.37, p=0.551)(Fig 7b).

Carapace width for crabs <25.9mm increased significantly in the unenhanced site (ANOVA, df=6, F=3, p=0.011) and the release site (ANOVA, df=6, F=88.71, p<0.00001) prior to the influx of wild recruits on the last two sampling dates (Fig 8a). Additionally, mean carapace width for crabs <25.9mm was significantly higher in the unenhanced site than in the release site (ANOVA, df=1, F=26.56, p<0.00001; Fig. 8a). Mean carapace width of crabs >25.9mm was not significantly different over time (ANOVA, df=8, F=1.35, p=0.229), but was significantly higher in the unenhanced site than in the release site (ANOVA, df=1, F=5.93, p=0.016) (Fig. 8b).

Size-frequency histograms for each sampling date show a cohort of juvenile crabs in the release site that decreases in number and increase in carapace width over time (Fig. 9). A cohort of wild recruits can also be observed on the last two sampling dates in both the release site and the unenhanced site (Fig. 9).

Regression analysis of mean crab density per basket for the distinct juvenile cohort observed in the size-frequency histograms showed that the number of crabs m^{-2} decreased exponentially within the release site (R^2 adj= 95.7%) to ~4 crabs m^{-2} (SE +/- 1.9015)(Fig. 10). The mean crab density per basket for the 'juvenile cohort' within the unenhanced site was shown to vary linearly (R^2 adj= 40.79%), decreasing only slightly over time (slope= -0.08 SE+/- 0.0325) with an average density of ~3 crabs m^{-2} (SE +/- 0.5432). Linear regression analysis of mean crab carapace width of the 'juvenile cohort'

showed an increase in size of 0.308mm day^{-1} SE+/- 0.0413 in the unenhanced site and by 0.4234mm day^{-1} SE +/- 0.0232 in the release site (Fig. 11).

Suction sampling

Crab ring suction sampling showed crab densities of ~6 crabs m^{-2} in the *Gracilaria* enhanced area of the cove and ~0.09 crab m^{-2} in unvegetated mud on July 3, 2007 (Fig.12). On July 16, 2007, crab densities were ~7 crabs m^{-2} in the *Gracilaria* enhanced region and ~0.1996 crabs m^{-2} in unvegetated mud (Fig. 12). Suctioning prior to the hatchery release found no crabs within the cove.

Genetic analysis

Of the 197 crabs collected in the release site on July 2nd, ~62% were genetically identified as hatchery-reared or wild (n=133). Approximately ~88% (CI +/- 0.059; n=108) of these crabs were hatchery-reared (Fig. 13). Of the crabs collected in the unenhanced site on July 2nd (n=33), ~91% (n=30) were genetically identified, ~27% (CI +/- 0.0797; n=8) of which were hatchery–reared (Fig. 13). Genetically identified crabs for July 2nd were assigned to size classes, rather than measured individually, due to the large number of crabs processed; 111 crabs were 4mm-8mm, 4 crabs were 8mm-15mm, and 1 crab was 15mm-25mm (Fig. 14).

Approximately 58% of the 38 crabs collected in the release site on July 31st were genetically identified, ~59% (CI +/- 0.105; n=13) were hatchery-reared (Fig. 13). Of the 29 crabs collected in the unenhanced site, ~52% were genetically identified and ~6.6% (CI+/- 0.064) of these were hatchery-reared (Fig. 13). Mean size of hatchery-reared crabs collected on this date was 23.9mm CW (SE +/- 2.465) and mean size for wild crabs was 58.6mm CW (SE+/- 8.56).

On Aug. 10th, ~89% and ~93% of crabs collected were genetically identified for the release site (n=95) and unenhanced site (n=56) respectively. Approximately 11% of those in the release site were hatchery-reared. There were no hatchery-reared crabs collected in the unenhanced site on Aug. 10th (Fig. 13). Mean size of hatchery-reared crabs was 26.6mm (CW)(SE+/- 1.93) and mean size for wild crabs was 12.7mm(CW) (SE+/- 1.25).

DISCUSSION

The measures of crab density in both sites over time suggest *Gracilaria* had a carrying capacity of ~4-8 crabs m^{-2} within the cove (Fig. 5). As expected, crab density was much higher in the release site than in the unenhanced site following the release. Crab density in the *Gracilaria* baskets in the unenhanced site remained constant over time, suggesting that crabs settled there until carrying capacity was reached. In contrast, capacity of the macroalgal habitat within the release site was likely exceeded and thus crab densities decreased until a supportable density was attained around 22 days.

Carrying capacity of *Gracilaria* may also be a function of crab size, so that as crab size increases within a site, carrying capacity decreases. In this case, the carry capacity within the release site, which contained large numbers of small hatchery-reared crabs, may have been reached early in the study, rather than after 22 days. Crab density within the release site then decreased, not because the carry capacity was exceeded, but because crab growth within the site continually lowered the carrying capacity. This theory also explains why crab density increased with the influx of wild recruits in early

August. Based on this, carrying capacity of *Gracilaria* could be as high as 20 crabs m^{-2} for juveniles around 7mm CW.

Crab ring suction sampling and basket sampling gave similar estimates of crab density within the *Gracilaria* enhanced area, while suction sampling estimates of crab density in the unvegetated areas of the cove were much lower. Basket samples could not be used to estimate density in the mud areas because baskets in the unvegetated areas were deliberately provided with *Gracilaria* as refugia to trap crabs for estimation of dispersal. These results suggest that the addition of *Gracilaria* and the release of the hatchery-reared juveniles significantly enhanced the carrying capacity of this lower Bay mud cove.

Changes in crab density and size within the cove were found to be largely due to crabs <25.9mm carapace width (Fig. 6, Fig. 8). Crabs of this size were not naturally present in high numbers until the beginning of recruitment in late July. Therefore, trends in crab density and size are likely to be due to the released hatchery-reared crabs. This is also supported by the presence of a distinct juvenile cohort within the release site that was absent at the unenhanced site (Fig. 9). Sizes of genetically identified crabs further indicate that the majority of crabs collected in this cohort were hatchery-reared. The disparity between sites was not observed once wild juvenile recruits appeared in the mud cove in early August, further indicating that the juvenile cohort observed in the release site was directly due to the addition of the hatchery-reared cohort and not because of a preference of juvenile crabs for one site over another.

Mean size of juvenile crabs appeared to differ between sites (Fig. 11) with mean size of crabs within the unenhanced site consistently higher than in the release site. One

possible explanation for this is that larger juvenile crabs dispersed at higher rates than smaller juveniles. Larger juvenile crabs may also have been able to make use of the slightly deeper unenhanced site, while smaller juvenile crabs remained restricted to the shallower release site. Because of a lack of data on the physical characteristics of the two sites no conclusions can be made based on this observation. However, these findings suggest that physical characteristics, such as water depth, may be important to consider when selecting areas for hatchery crab release.

It is not clear what proportion of the decrease in crab density within the release site was due to predation versus emigration of juveniles from the cove. The presence of hatchery crabs in the unenhanced site indicates that at least some emigration did occur. Post-settlement dispersal has been shown to occur in response to high density in seagrass habitats. Reyns and Eggleston (2004) found planktonic density of first juvenile instars increased dramatically at benthic densities of ~8-10 crabs m^{-2} in seagrass. The observation of a carrying capacity suggests that hatchery crabs may have emigrated from the cove in a density-dependent fashion.

Crab abundance was expected to correlate with algal volume, but no significant trends were present in the data. Nevertheless, crab density did appear to increase with algal volume (Fig. 4). There are several possible explanations for the lack of significance. The volumes of *Gracilaria* used in this study may not have differed sufficiently to create a significant difference in crab abundance. Alternatively, algal volume may not be a sufficient predictor of crab abundance within an algal mat. Other variables, such as the amount of bottom coverage or density of an algal mat, may be important in determining the number of individuals *Gracilaria* can support. Associations

of juvenile blue crabs are complex in seagrass beds. Crab abundance in seagrass has been shown to depend on both seagrass patch size and shoot density (Hovel et al., 2002; Hovel & Fonseca 2005) and vary temporally with seasonal changes in predators (Hovel and Lipcius, 2001). Further study of the algal habitat dynamics will be needed to understand how juvenile crab abundance varies in *Gracilaria*.

The finding that some hatchery-reared juveniles remained in the release cove during the entire 43 day period was significant. Previous releases in the lower Chesapeake Bay have had limited success in tracking hatchery-reared individuals post-release. The large number of hatchery-reared individuals remaining in the release site during this study was likely due to (1) increased survival and settlement due to enhancement along with (2) an improved ability to identify hatchery-reared crabs.

Numerous studies have demonstrated that settlement and survival of small juvenile blue crabs (<20mm CW) is higher in structured habitat than in unstructured habitat (Orth and van Montfrans 1987; Williams et al., 1990; Thomas et al., 1990; Pile et al., 1996). Results from this study suggest that enhancement of the release site with *Gracilaria* spp. decreased emigration of hatchery-reared crabs by providing structured habitat. The structural refuge provided by *Gracilaria* may have also increased survival of juvenile crabs, following the release, by reducing predation. Juvenile crab survival has been shown to be higher in *Gracilaria* spp. than in both mud and seagrass (Johnston et al., unpublished). Availability of structured habitat within release sites will likely be an important factor to consider when developing release strategies.

The apparent high success of this release may have also resulted from the use of genetic markers to identify hatchery-reared crabs. Previous releases in the lower

Chesapeake Bay have used coded microwire tags to track individuals post-release. While this is an effective method for tagging blue crab juveniles, it has disadvantages (Davis et al., 2004b). Microwire tagging can result in mortality of tagged individuals and tags can be lost after successive molts (Davis et al., 2004b). Further, microwire tagging is labor intensive, limiting the number of crabs that may be released at one time. The use of genetic markers to identify hatchery-reared individuals in this study likely increased survival of released crabs by eliminating the need for pre-release handling and tagging. Because microwire tags can be lost during molting, this method also may have increased the ability to identify hatchery-reared individuals over time.

Results from this study show that hatchery-reared crabs remaining within the release site nearly quadrupled in size over the sampling period. Mean size of hatchery-reared crabs increased from ~7 mm on the release date to ~27mm on Aug. 10th. Released crabs were thus likely able to reach a size refuge from predation (Pile et al., 1996). This rate of growth (~.4mm day^{-1}) in the hatchery-reared cohort also suggests that a portion of hatchery-reared individuals may have been able to reach maturity and mate before overwintering in late November. Because success of stock enhancement efforts is ultimately determined by the ability of released crabs to grow to sexual maturity, these results are extremely encouraging.

The presence of a large cohort of wild juveniles in early August demonstrates that *Gracilaria* spp. not only functions as a nursery habitat, but is also selected by individuals recruiting and dispersing into the York River estuary, thus playing an important role in blue crab ecology. The results of this study demonstrate the ability of *Gracilaria* to support high densities of juvenile of blue crab and to function as an alternative nursery

habitat. However, many characteristics of benthic macroalgae, including occurence in low oxygen environments, seasonal variability, and ability to drift, suggest *Gracilaria* may be a less suitable habitat than traditional seagrass beds. Given the challenges facing the blue crab, *Callinectes sapidu, s* in the Chesapeake Bay, further study of role of macroalgal habitats clearly will be needed.

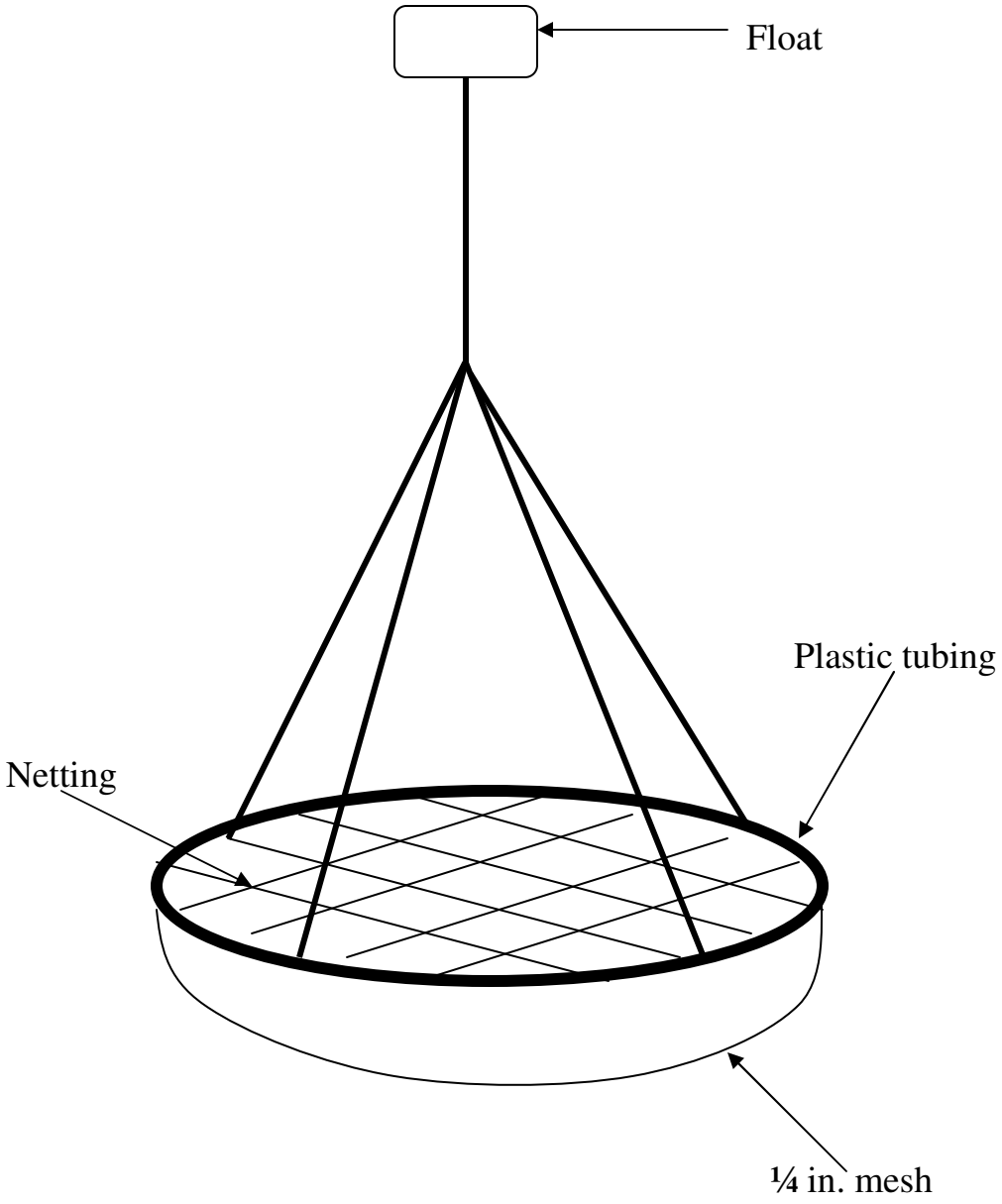

Fig. 1: Basket apparatus used for field sampling. Different volumes of *Gracilaria* spp. were contained in the pocket created by the ¼ in. mesh. When deployed, the basket laid flat on the sediment surface.

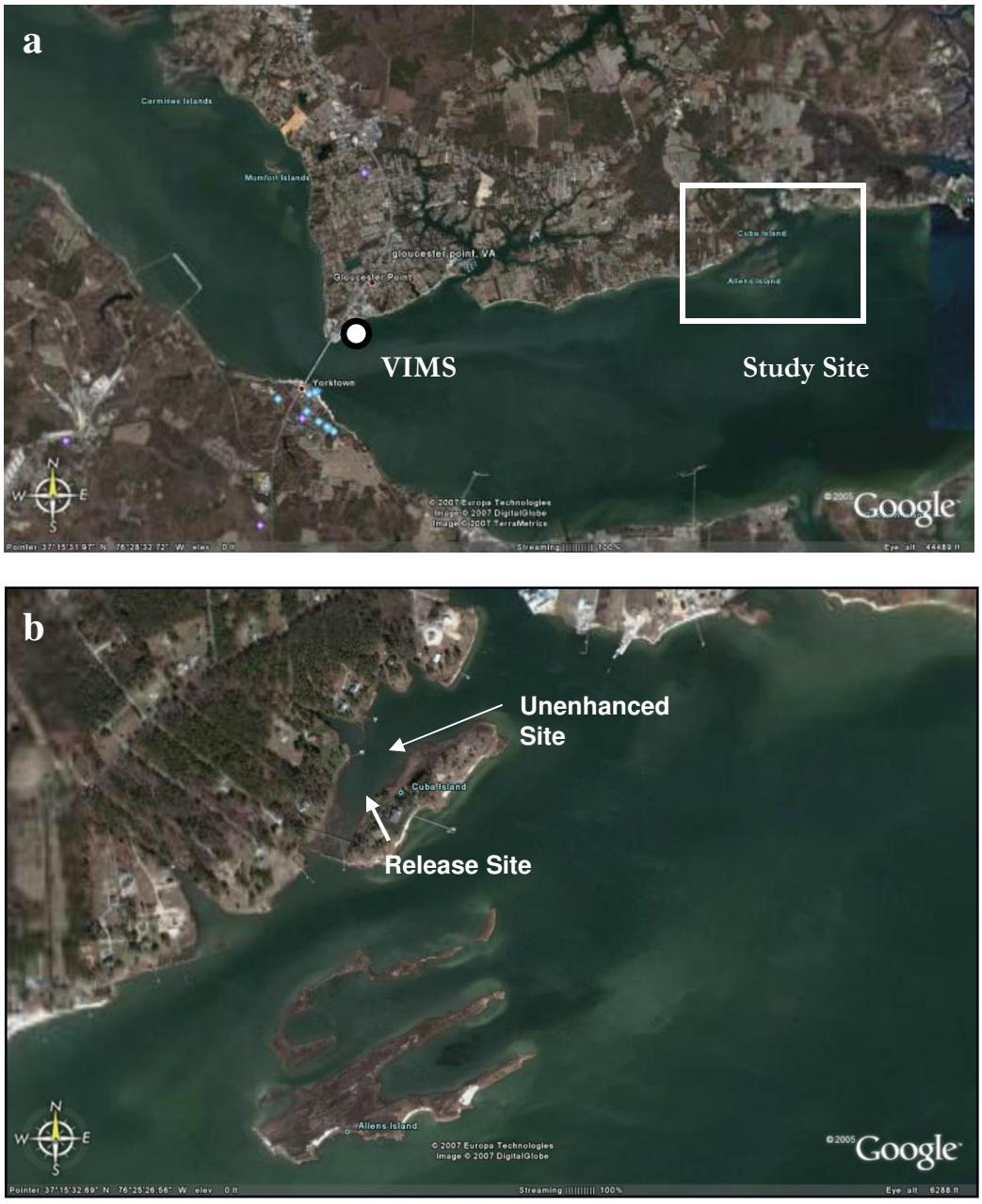

Fig. 2: Map of study location (a) relative to the Virginia Institute of Marine Science (VIMS) and (b) showing the two different sampling regions.

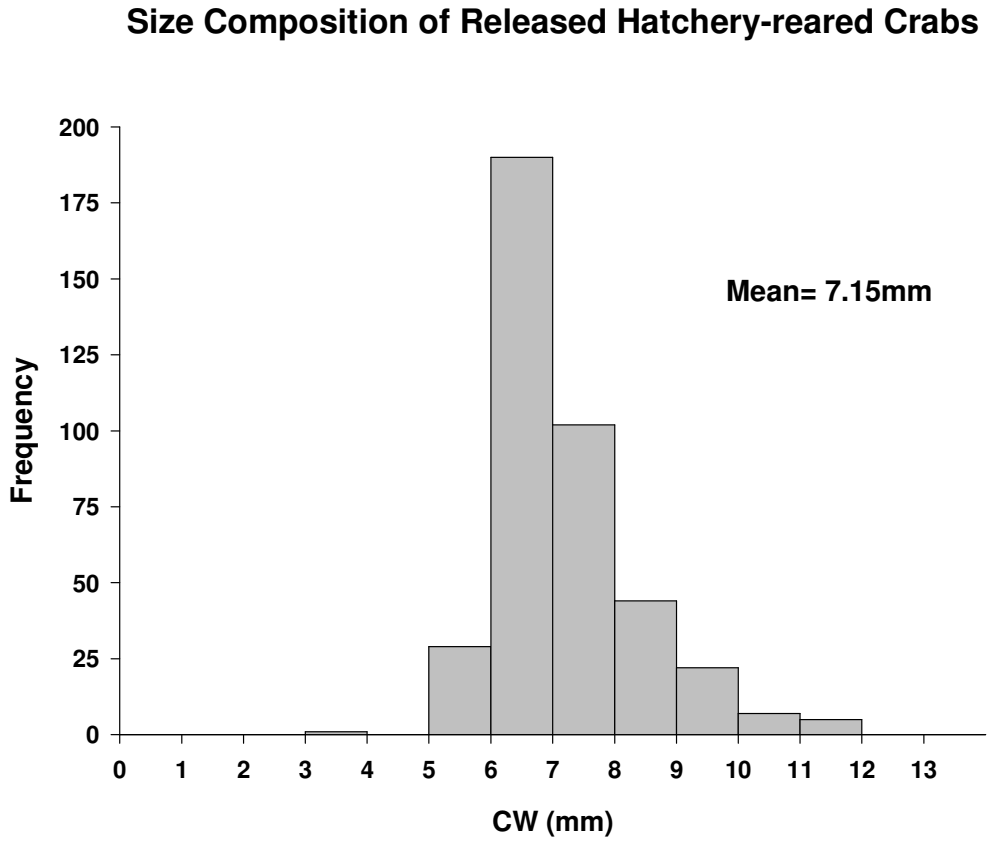

Fig. 3: Size distribution of hatchery-reared crabs from a subsample of 400 crabs taken on the release date. Crabs ranged from 3.1mm to 11.9mm carapace width; mean size was 7.15mm (SE +/- 0.0581) carapace width.

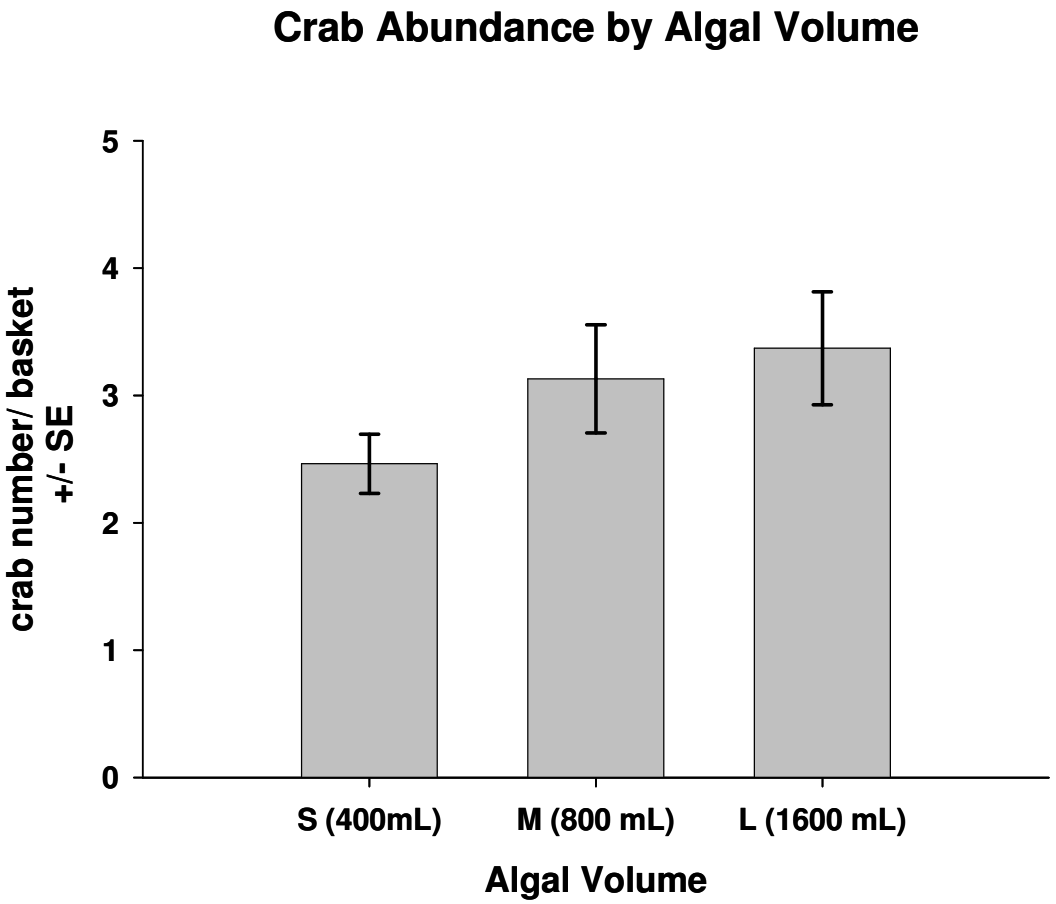

Fig. 4: Crab abundance (mean +/- SE) of baskets containing different algal volumes. Number of crabs did not vary significantly (ANOVA, df=2, F=1.54, p=0.216).

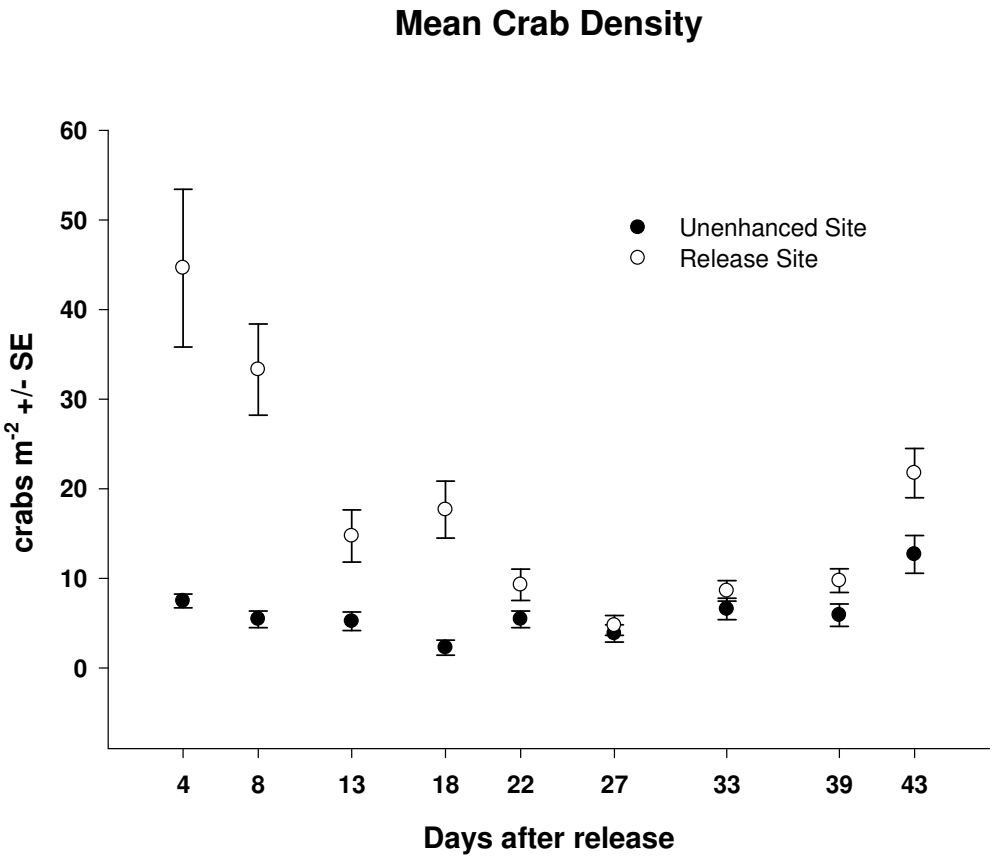

Fig. 5: Variation in crab density (mean crabs m^{-2} +/- SE) over the study period in the release site and unenhanced site

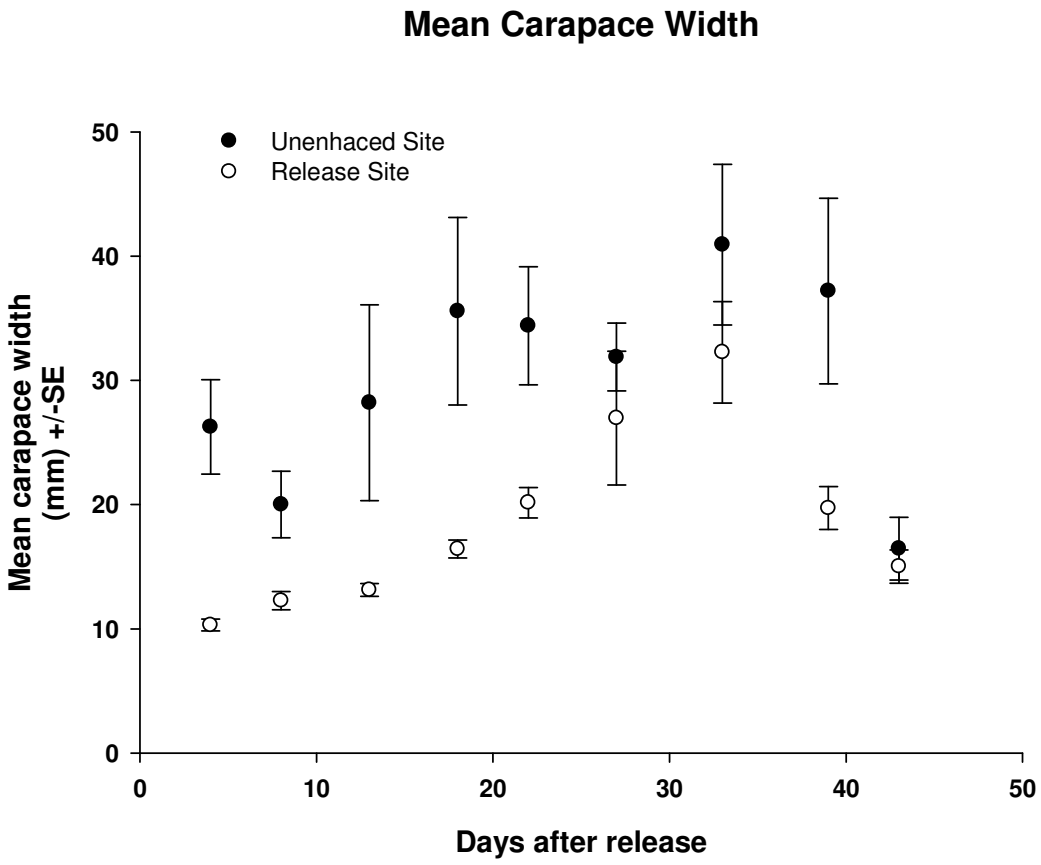

Fig 6: Variation in crab carapace width (CW) (mean +/-SE) over the study period.

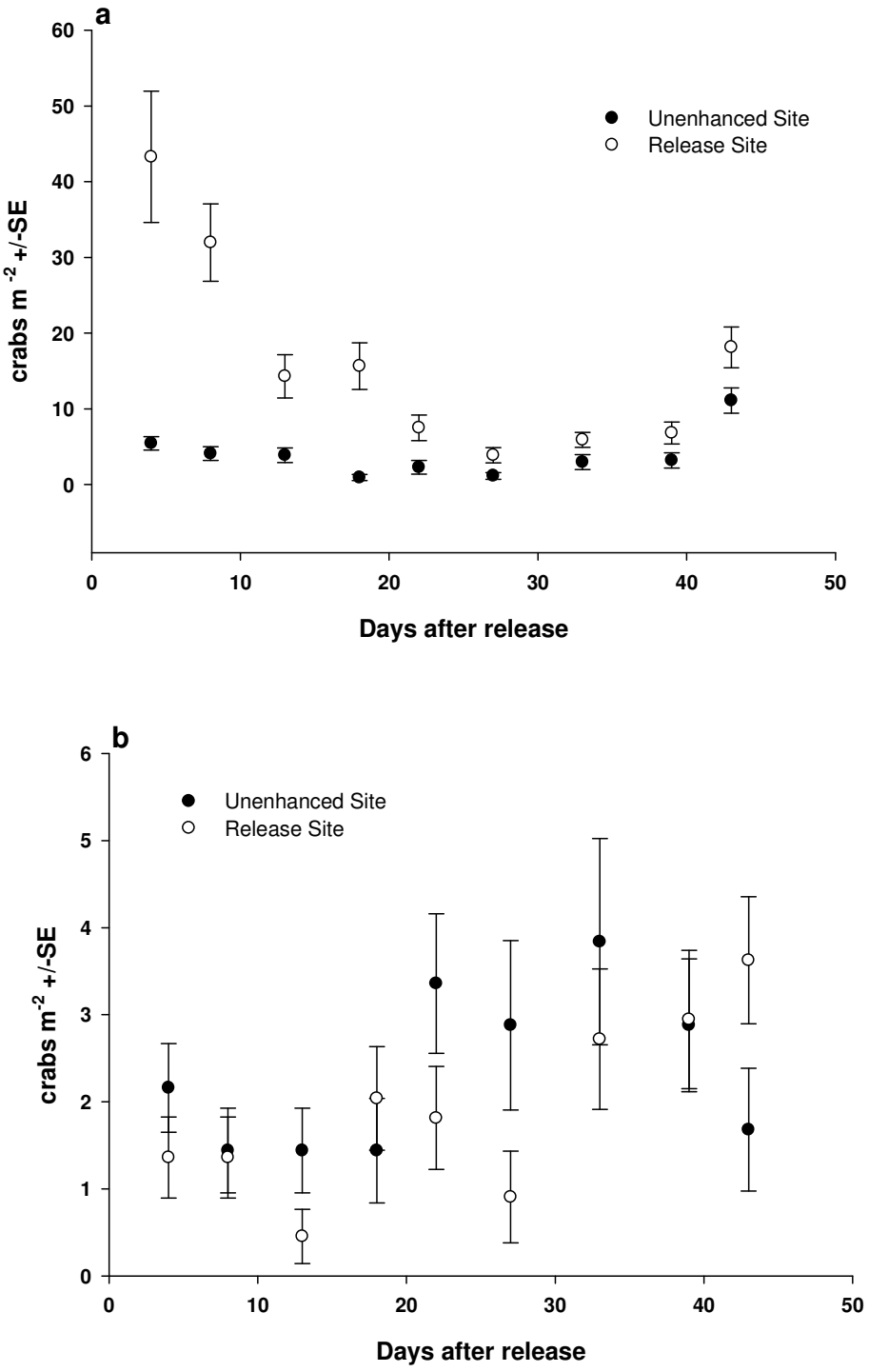

Fig. 7: Crab density (mean +/-SE) (a) for crabs <25.9mm carapace width was significantly higher in the release site for the first 18 days of the study (ANOVA, df=1, F=10.5, p=0.018) but did not differ for the period 22-43 days (ANOVA, df=1, F=0.37, p=0.551) (b) for crabs >25.9mm did not differ significantly between sites (ANOVA, df=1, F=1.97, p=0.198)

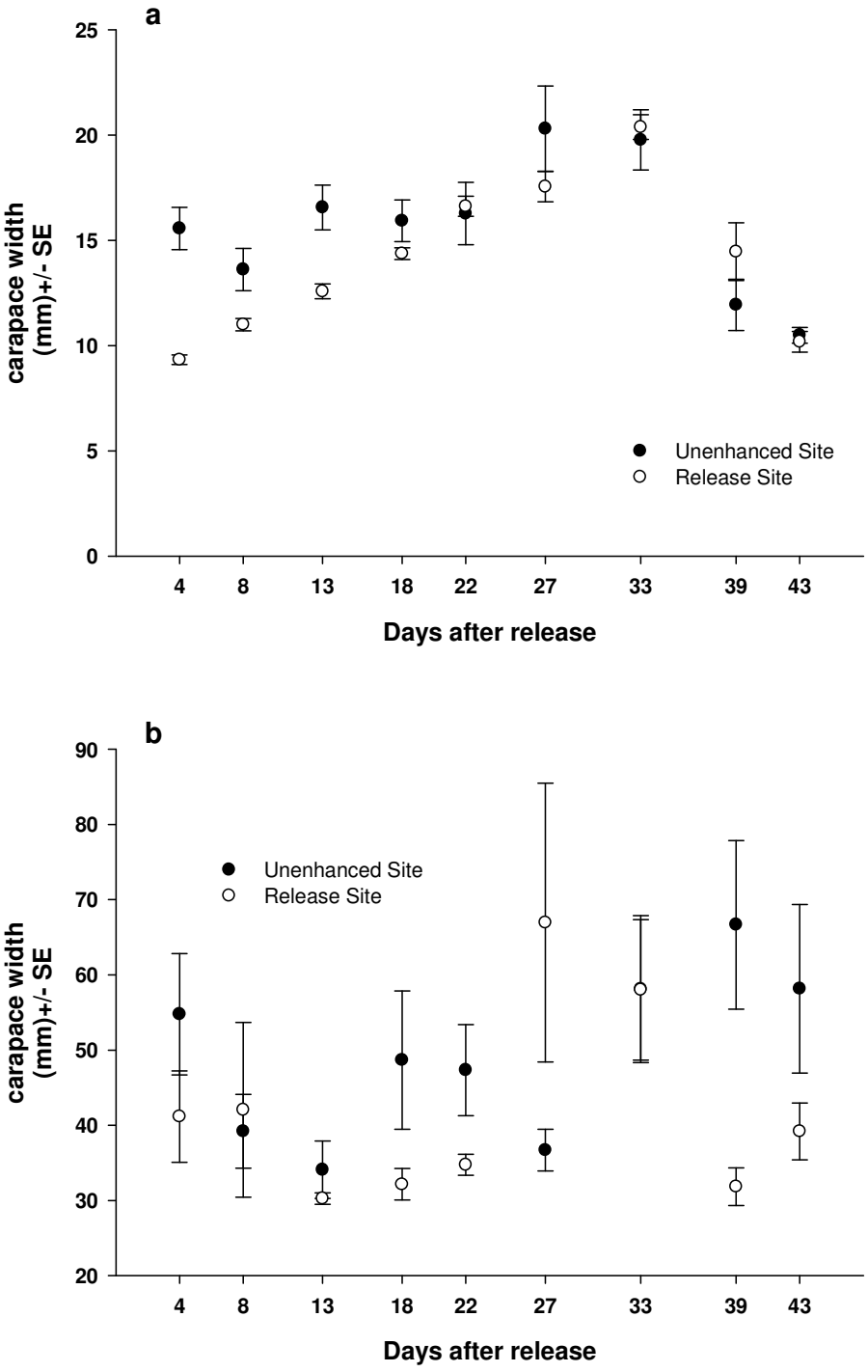

Fig. 8: Crab carapace width (mean +/-SE) (a) for crabs <25.9mm CW increased significantly in the release site (ANOVA, df=6, F=88.71, p<0.0005) and the unenhanced site(ANOVA, df=6 F=3 p=0.011) prior to the influx of recruits on the last two days of sampling (b) for crabs >25.9mm did not vary significantly over time (ANOVA, df=8, F=1.35, p=0.229)

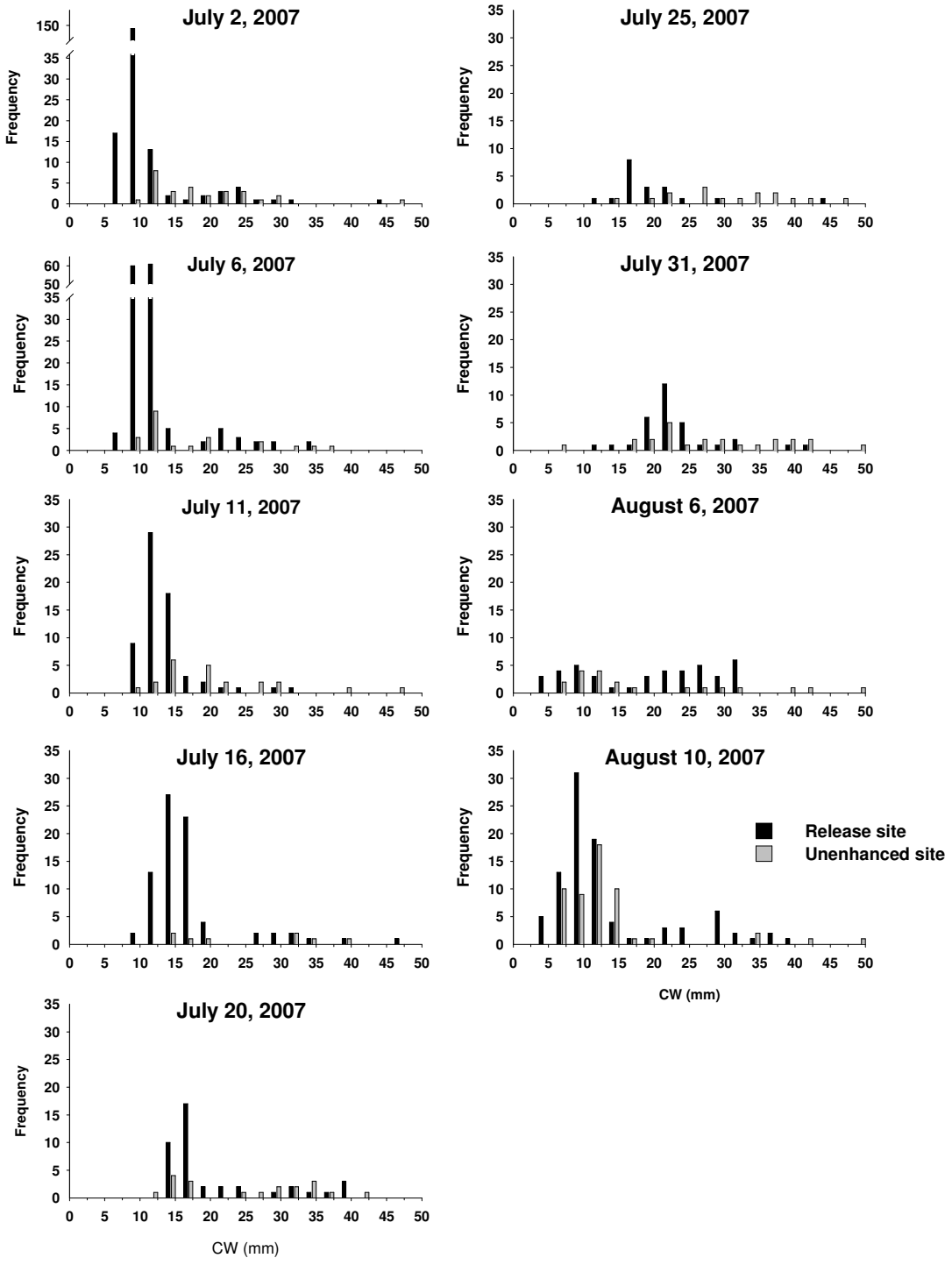

Fig 9: Size (carapace width) frequency in each site on each sampling date.

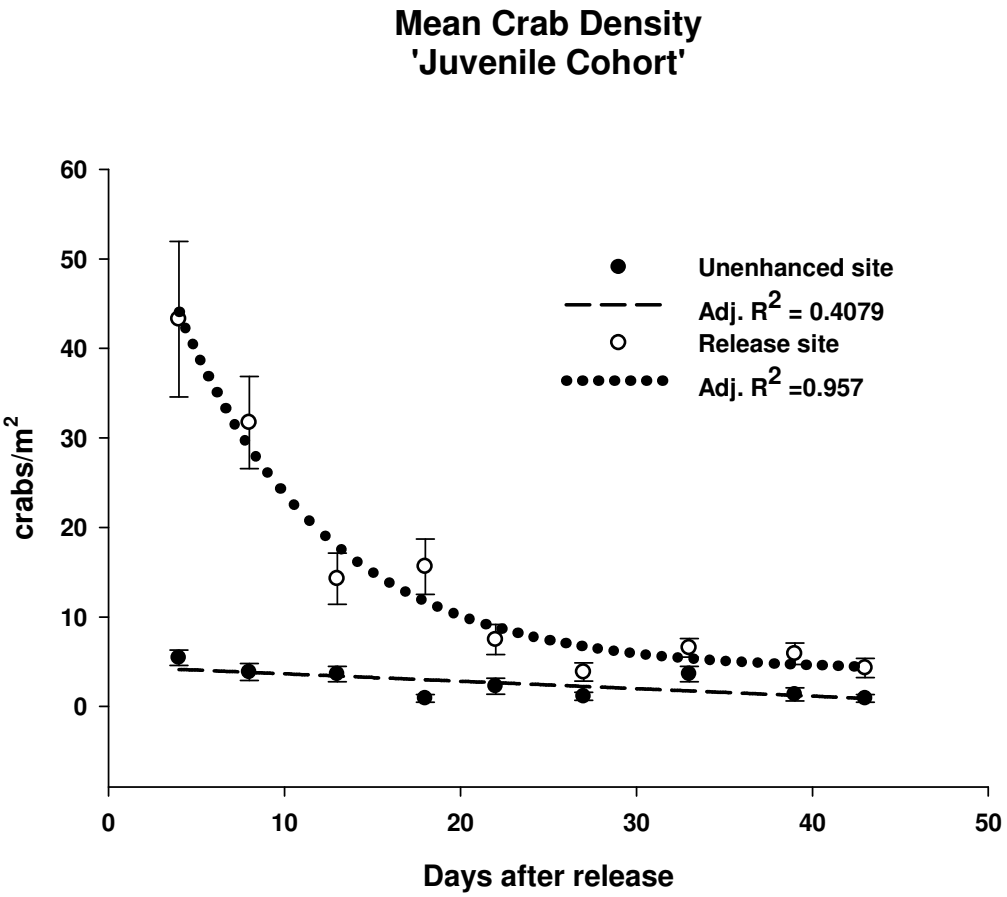

Fig. 10: Crab density (mean +/-SE) for the juvenile cohort observed in size-frequency histograms

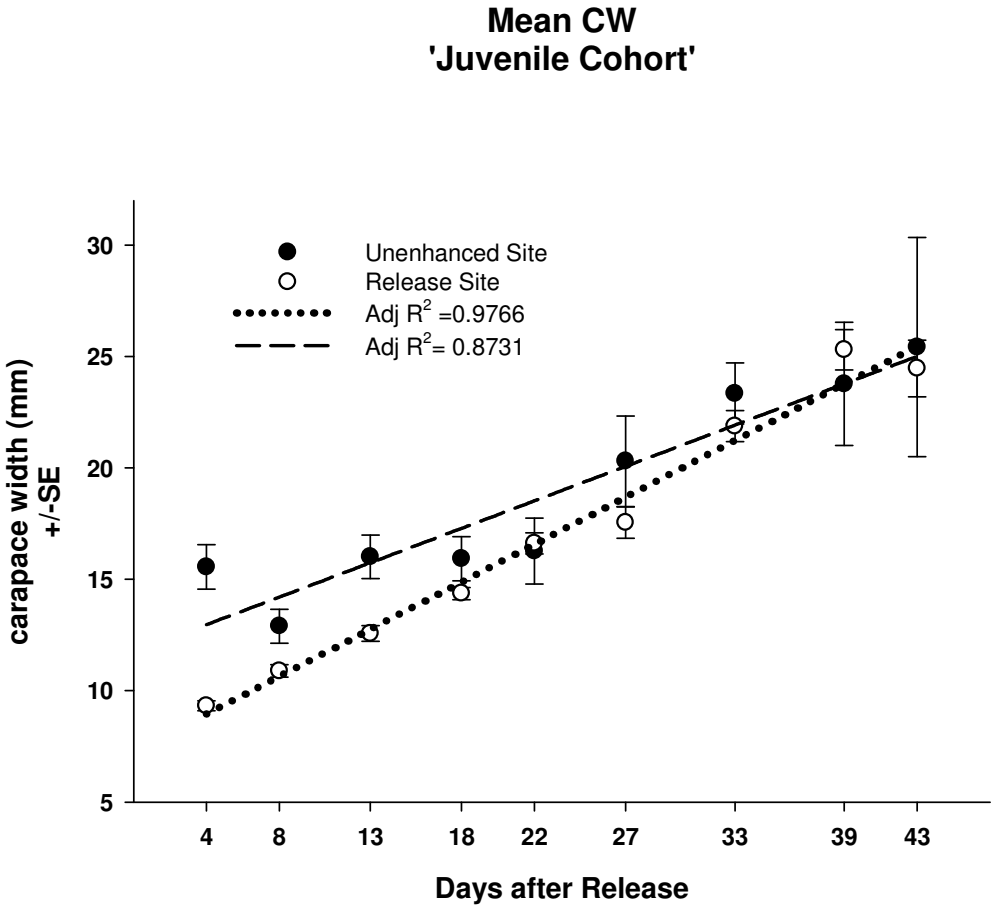

Fig 11:Crab carapace width (mean +/-SE) for the juvenile cohort observed in size-frequency histograms.

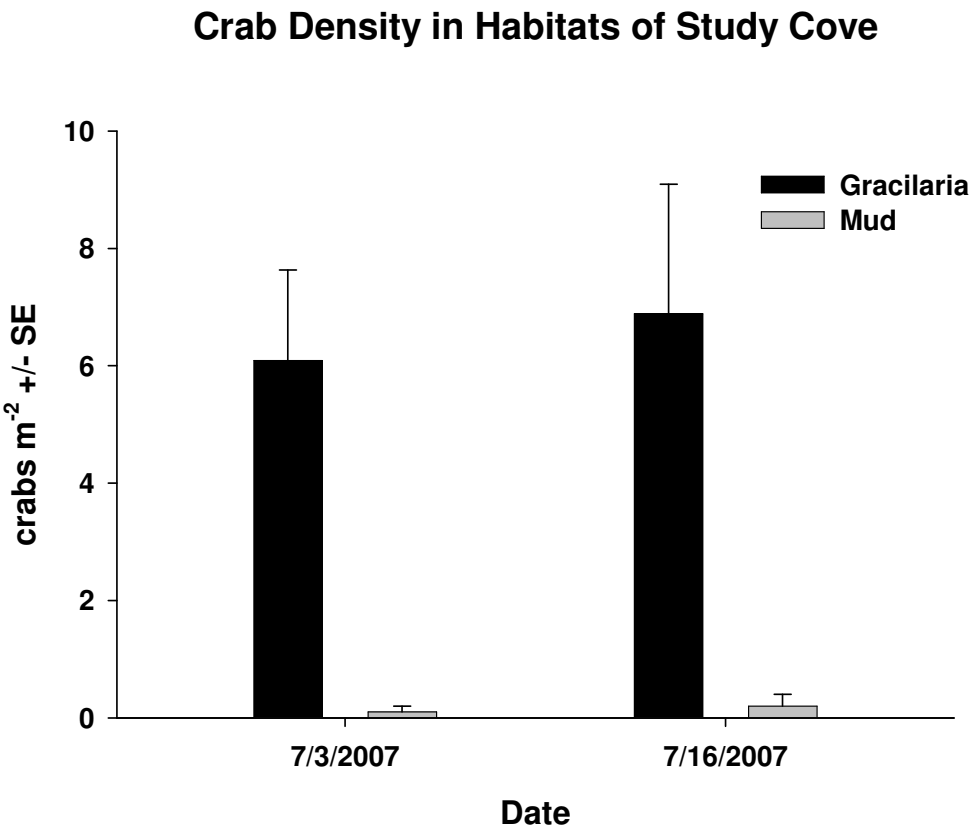

Fig 12: Crab density in *Gracilaria* and mud habitats within the study site from crab ring suction sampling conducted on July 3rd and July 16th .

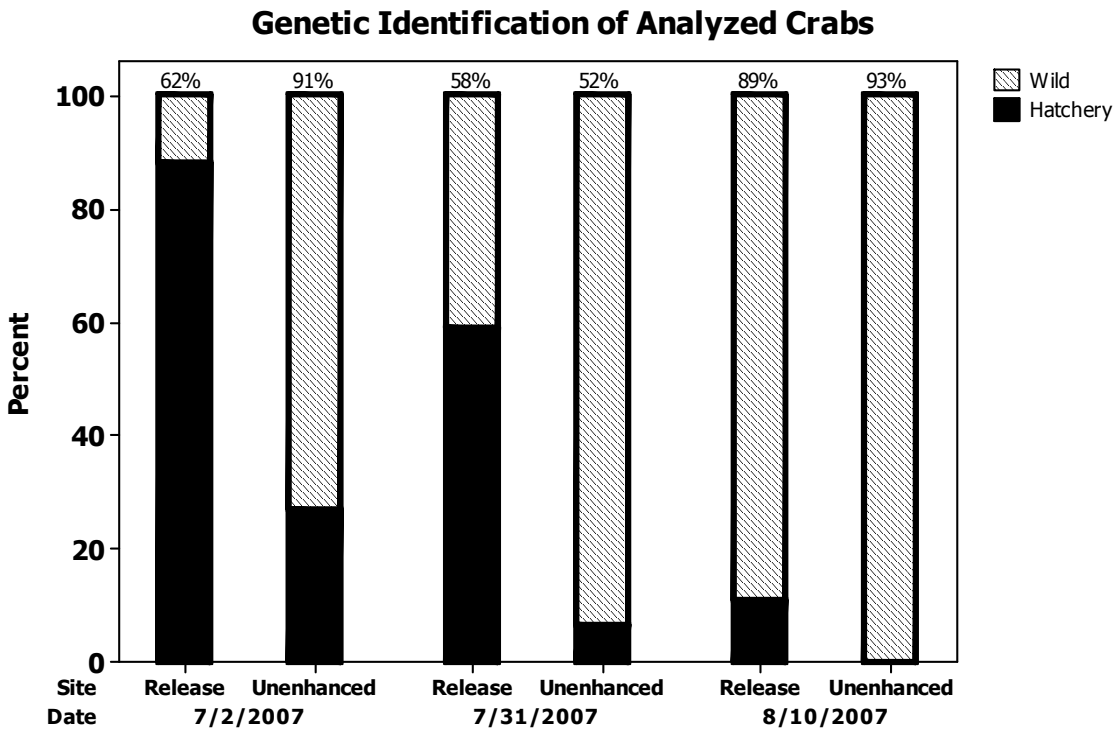

Fig. 13: Percentage of the genetically identified crabs that were either hatchery-reared or wild. Bar labels represent the percentage of crabs genetically identified out of the total number of crabs collected on a particular date in a particular site.

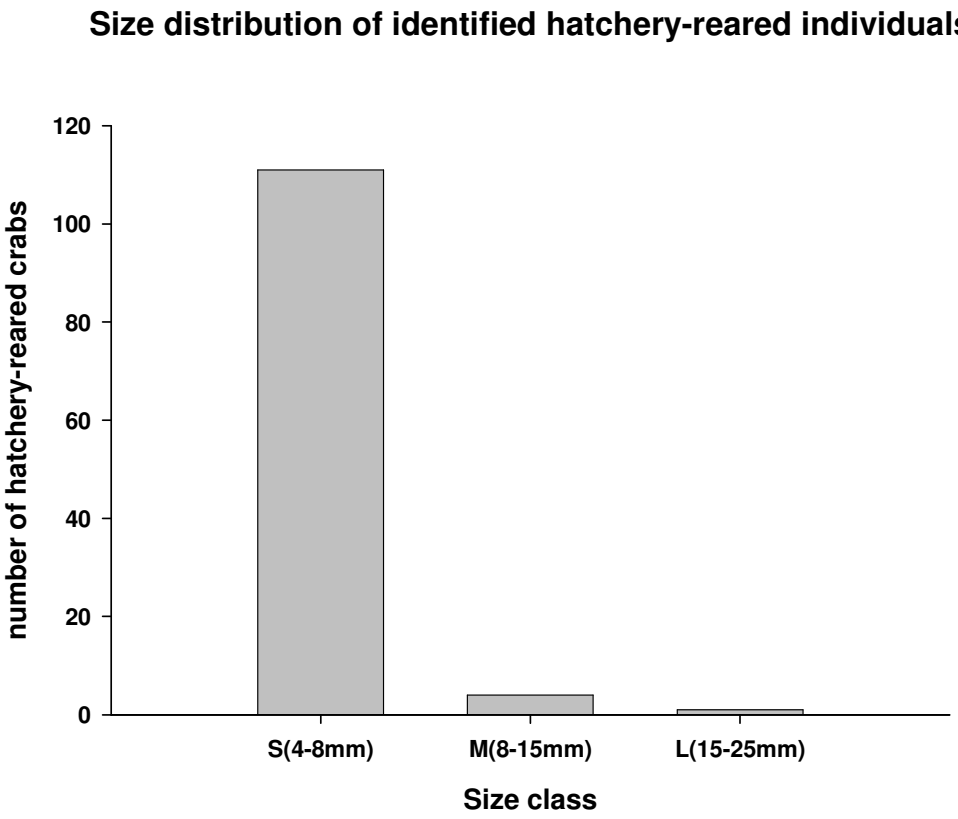

Fig. 14: Size distribution for genetically identified hatchery-reared individuals collected on July 2, 2007. Size classes small, medium, and large contained n==111, n=4, and n=1 respectively.

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